Conclusion: this study suggests that synovitis is a common feature in painful knee osteoarthritis, associated with more severe chondropathy, but it also suggests that synovitis could be considered as a predictive factor of subsequent chondrolysis.

PP6

MACROPHAGES - ACTIVATED BY TGF-β PROMOTE OSTEOPHYTE FORMATION

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Purpose: Osteophytes (new formation of cartilage and bone) are important features of osteoarthritis. Growth factors like TGF-β have been shown to induce osteophyte formation through outgrowth of periosteal cells. Macrophages are important producers of a range of growth factors and as they cover the inside of diarthrodial joints, they may well form an important source of growth factors involved in osteophyte formation. In the present study, we investigated whether macrophages have an intermediate role in osteophyte formation.

Methods: In vitro, the interaction between murine macrophages and mesenchymal cells (precursors with chondrogenic potential) were studied using a Transwell system. Murine peritoneal macrophages, brought into the upper compartment, were cocultured with C3H10T1/2 mesenchymal cells present in the lower compartment. Spheroid neo cartilage formation was quantified under the microscope after staining with May Grunwald Giemsa. Various concentrations of TGF-β were added to macrophages. In vivo. synovial lining macrophages were selectively depleted by injection ol clodronate-laden liposomes prior to triple injections of 20 ng of TGF-b at alternate days. Total knee joint sections were taken at day 7 after the last injection and stained with safranin-O.

Results: Clustering and spheroid formation of C3H10Tl/2 was induced by TGF-β concentrations above 1 ng/ml. Lower concentrations (0.75 and 0.5 ng/ml) were ineffective. However, in the Transwell system, in the presence of macrophages, *0.5* ng/ml TGF-β was very effective in generating large spheroids, suggestive of macrophage-derived (co)factors. Using a specific ELISA, we found that in the co-culture supernatants, TGF-β concentration were lower in the presence of macrophages, making autoinduction of TGF-β unlikely and pointing to generation of other growth factors involved in spheroid formation. In addition, the contribution of macrophages to osteophyte formation was studied in vivo. Triple injections of 20 ng TGF-β into normal murine knee joints showed that at day 7 after the last injection significant osteophyte formation was observed at the lateral and medial site of patella and femur. Strikingly, removal of synovial lining macrophages prior to triple injection of TGF-β resulted in a drastic reduction (decrease - 80%) of osteophyte formation.

CONCLUSION: This study suggests that macrophages are crucial intermediates in osteophyte formation induced by TGF-β and apart from TGF-β, other macrophage derived (growth) factors promote this process.

PP7

A NOVEL PROTEIN-BINDING SITE (AGRE) IN HUMAN COLLAGENASE-3 PROXIMAL PROMOTER REGION IS INVOLVED IN REPRESSION OF TRANSCRIPTION

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Activation of collagenase-3 (coll-3) transcription in osteoarthritic (OA) cartilage involves proteins binding at sites such as AP- 1 and PEA-3. In this study, we have identified a novel protein-binding site, AAAAGAAAAAG (bp -117 to -127 in the coll-3 promoter), consisting of two copies of the pentanucleotide AAAAG separated by one nucleotide. This site was designated AGRE (AG-Rich Element). We demonstrate, for the first time that this site binds proteins that repress basal coll-3 transcription.

Method and Results: Human OA chondrocytes as well as four cell lines (COS-7, HEp-2 SW1353 and HeLa) were transfected with a plasmid consisting of the first 133 bp of the coll-3 promoter (containing the TATA box, the AP-1, PEA-3 and AGRE sites), and its AGRE mutated or deleted derivatives. Data revealed that the absence of a functional AGRE site, following its mutation or deletion, resulted in a significant increase in the coll-3 basal transcription in OA chondrocytes (183%, $p<0.02$; 221%, $p<0.03$, respectively). Deletion of the AGRE site also showed a 2-fold increase in the cell lines. No effect was found when chondrocytes were treated with coll-3 inducers, IL-1b and TGF-b. Two specific protein-AGRE binding complexes were detected by EMSA: a slower (complex #1) and a faster (complex #2) migrating complex. Their appearance seems to depend on the physiological state of the cell. Indeed, normal chondrocytes, synovial fibroblasts and the four cell lines showed only the complex #1. In OA chondrocytes, the complex number appearance discriminates two subgroups: the low-OA chondrocytes, showing low coll-3 basal levels and high inducibility of IL-lb stimulation (complex #1), and the high-OA chondrocytes, with high coil-3 basal levels and low IL-1b inducibility (complex #2). UV cross-linking revealed the presence of two major proteins with different molecular weight in complexes #1 and #2: 35 and 70 kDa for complex #1, and *45* and 100 kDa for complex #2. As above, the protein abundance was not influenced by either IL-1b or TGF-b.

Conclusion: We report a novel negative regulatory element in the coll-3 promoter. The presence of different protein-binding complexes is affected by the metabolic state of the cells. These findings suggest that the AGRE site plays a rate limiting step in coll-3 production, and may represent a likely target in the OA pathophysiology process.

PP8

COMPARISON OF HOMEOSTASIS OF THE EXTRA-CELLULAR MATRIX BY PHENOTYPICALLY STABLE CULTURED HUMAN CHONDROCYTES FROM NORMAL AND OSTEOARTHRITIC CARTILAGE OF THE SAME KNEE

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Objective: Synthesis and accumulation of the ECM is regulated by locally produced growth factors such as IGF and TGF-β. Turnover and degradation of the matrix is dependent on the responsiveness of the chondrocyte to catabolic cytokines such as IL-1α/β and TNF. The function of auto/paracnne anabolic (IGF-2/IGF-Re2) and of catabolic (IL-lα and B/IL-1 -Re) pathways in the homeostasis of the extracellular matrix (ECM) of nonnal and osteoarthritic (OA) articutar cartilage cells was investigated.

Methods: Phenotypically stable human articular cartilage cells were obtained from normal and OA cartilage of the same knee and maintained in culture in alginate beads over 1 week to reach

equilibrium in accumulated CAM compounds. After liberation of the cells from the alginate beads, the levels of CAM aggrecan, type II collagen and fibronectin, of intracellular IGF-2, IL-lα and β and of their respective plasma membrane-bound receptors: IGF-Re2, IL-1-Re I and the decoy receptor IL-1-Re2 were then assayed using flow cytometry.

Results: When compared with cells obtained from normal tissues, OA chondrocytes expressed significantly higher intracellular IGF-2 levels and plasma membrane-bound IGF-Re2. At the same time, significantly higher intracellular IL-la and b levels and upregulated plasma membrane-bound IL-1-Re1 were observed. Plasma membrane-bound IL-1-Re2 decoy receptor was down regulated in OA chondrocytes. The levels of CAM aggrecan, type II collagen and fibronectin were significantly reduced in the chondrocytes obtained from pathological tissue.

Conclusion: Chondrocytes from OA cartilage show an enhanced capacity to produce ECM macromolecules. However, the same cells have increased catabolic signaling pathways. As a consequence of this increased IL-1 activity and the reduced amounts of IL-I-Re2 decoy receptor, less of the produced ECM macromolecules will remain in the CAM of the OA chondrocytes.

PP9

THE EFFECTS OF CALCIUM PENTOSAN POLYSULFATE IN SYNOVIAL PATHOLOGY, NITRIC OXIDE SYNTHASE EXPRESSION AND HYALURONAN PRODUCTION IN AN OVINE MODEL OF OSTEOARTHRITIS

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The present study was undertaken to determine the effects of the potential structure-modifying anti-osteoarthritis drug, calcium pentosan polysulfate (CaPPS) on the synovial pathology and expression of iNOS by synoviocytes and chondrocytes from osteoarthritic (OA) joints. The ex vivo and in vitro biosynthesis of hyaluronan (HA) by cultured synoviocytes was also studied.

Methods. Twelve of 18 aged ewes were subjected to meniscectomy to induce OA. Six of the OA ewes were orally administered 20mg CaPPS/kg twice weekly for 24 weeks, after which all 12 OA and 6 non-operated controls (NOC) were killed. Supra-patellar synovia and cartilage were removed to 10% (v/v) neutral-buffered formalin for histology and the remaining synovium digested with trypsin and collagenase. The released synoviocytes were cultured and labeled with ^{3}H-acetate at the second passage. Synoviocytes were also cultured with CaPPS. 3H-labelled HA was quantitated by differential digestion with streptomyces hyaluronidase and sized by gel permeation chromatography. Histological sections were stained with haematoxylin and eosin and scored by a blinded observer for intimal hyperplasia and cellularity (IC), cellular infiltrate (CI), subintimal Fibrosis (SF) and vascularity. Inducible nitric oxide synthase (iNOS) was immunolocalized using published methods.

Results. CaPPS, when added to synoviocytes in culture, significantly increased the biosynthesis of high MW HA by over 500% regardless of whether the cells were from NOC or OA sheep. Oral CaPPS treatment increased the biosynthesis of HA by OA synoviocytes. Scores of CI, SF and IC for OA synovia were elevated relative to NOC ($p < 0.05$). Dral CaPPS significantly reduced scores for SF and IC ($p < 0.05$). expression of iNOS was increased in OA synovia but not in the corresponding tissues from OA and CaPPS treated animals.

Conclusions. Six months of biweekly administered CaPPS mitigated the synovial inflammatory response, iNOS expression and enhanced the biosynthesis of HA both ex vivo and in vitro in OA joints, thus supporting its classification as a SMOAD.

PP10

THE CARTILAGE/BONE INTERFACE IS PERMEABLE TO SALINE UNDER PHSYIOLOGIC ARTICULAR PRESSURES

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Aim: To test the hypothesis that the interface between cartilage and bone may be permeable to saline under the large loading pressures that prevail in normal joints.

Methods: Eight mm "buttons" of cartilage and subchondral bone were trephined from the normal humoral heads of pregnant ewes, adult dogs, and immature pigs. Each specimen was sealed at the base of a 5 ml plastic syringe leaving ~20mm^2 of cartilage surface under 2 ml of normal saline. Reinforced plungers were then inserted and loaded to generate pressures of 1.5 mPa (15 atmospheres), and rates of throughflow were quantified precisely in appended glass capillaries:

Results:

Species	n	flow ± SEM (m/min)
Sheep	24	0.016 ± 0.002
Dog	10	0.017 ± 0.003
Pig	8	0.348 ± 0.024

Conclusions: All individual specimens were permeable under this normal loading pressure. The higher permeability of porcine specimens is attributed tentatively to a difference in maturity rather than species. Consistent permeability to saline implies: 1. a potential path for transport of metabolites between marrow microvessels and deep chondrocytes, 2. an invasion route for pathologic cytokines from synovial effusions into the subchondral space, and 3. a possible explanation for the anatomic "tidemark" in normal cartilage. If, as we suspect, the barrier between calcified and uncalcified cartilage is semipermeable, then larger constituents of degraded chondrocytes may filter out there to leave the tidemark as a potentially immunogenic depot of apoptotic debris.

PP11

THE CARTILAGE DEGENERATION AFTER IMMOBILIZATION IN *p53 (+1+)* AND p53 *(-I-)* MICE KNEES

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Objectives: We investigated the cartilage degeneration and the frequency of apoptotic cells in p53 *(+/+)* and *p.53 (-I-)* mice after immobilization.

Materials and Methods: Right knees of male p53 *(+/+)* mice and p.53 *(-I-)* mice (8-week-old) were immobilized in full extension with bandage for 0, 2, 4, 8 and 12 weeks. Histologically, cartilage tissues were stained by safranin 0. Apoptotic cells were confirmed by TUNEL staining on the sections of knee joints. Total RNAs of chondrocytes obtained from non-immobilized or immobilized mice knees were analyzed quantitatively with using RT-PCR method. Amplification of generated cDNA was performed with specific primer of *p53.*

Result: In *p53 (+/+)* mice, histological study revealed loss of metachromasia in the articular cartilage at 2 weeks, fissuring at 4 weeks, and loss of cartilage layers 8 or 12 weeks after immobilization. On the other hand, in *p53 (-/-)* mice, cartilage layer was maintained not to degenerate. Apoptotic cells were observed in articular cartilage, meniscus, trabecular bone and growth plate in knee joints of *p.53 (+1+)* mice. Apoptotic cells in articular cartilage detected at more than 4 weeks after immobilization were